Prevalence of vitamin D deficiency and factors associated with vitamin D deficiency among 13-17 years old adolescents in Coimbatore

Master of Public Health Integrating Experience Project

Research Grant Proposal Framework

by

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List of Abbreviations:

1. World Health Organisation(WHO)
2. Estimated Average Requirement(EAR)
3. Body Mass Index(BMI)
4. Metabolic Equivalents(MET)
5. Institute of Medicine(IOM)
6. American Academy of Pediatrics (AAP)

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Executive Summary

Vitamin D deficiency is a global health problem that is highly prevalent in many regions of the world including India. Vitamin D deficiency may lead to several diseases, including nutritional rickets, osteomalacia, skeletal deformities, increased risk of hip fractures, and increased risk of preeclampsia among pregnant women. The factors associated with Vitamin D deficiency include lack of sun exposure, inadequate diet, low level of physical activity, second hand smoke exposure, soft drink consumption, older age, female gender and lower socio-economic status.

Few studies have been conducted to explore the prevalence of vitamin D deficiency and the factors associated with the deficiency among adolescents in India. As adolescence is accompanied by the rapid growth of bones and sexual development, vitamin D is particularly important in this age group. The proposed study will explore the prevalence of vitamin D deficiency and the associated factors among the adolescents aged 13 to 17 years in Coimbatore, Tamil Nadu, India. A cross-sectional survey will include blood sample collection in order to assess the 25-hydroxy vitamin D levels in blood (outcome) and the administration of a self-administered questionnaire that will measure sun exposure, vitamin D in diet, age, exposure to second hand smoke and soft drink consumption. The height and weight of the participants will be also measured in the survey. The data on the intake of supplements and socio economic status of adolescents will be obtained from their parents.

The data will be collected from 864 students across 10 schools in Coimbatore city. The multi-stage cluster sampling will be used to sample the participants. Logistic regression will be done for analysis. Simple and multiple regression models will be used. The approval for the study was obtained from the Institutional Review Board of the American University of Armenia. The proposed budget for the study is 17,760 USD. The proposed study duration is five months.

Introduction

Vitamin D deficiency is an important health concern worldwide. It has been estimated that about a billion people around the globe are vitamin D deficient or insufficient.[1]

Vitamin D is synthesized from the UVB rays obtained from the sun.[2] Some of the major food sources of Vitamin D include salmon and cod liver oil.[2] The two major forms of vitamin D include vitamin D2 (ergocalciferol) and vitamin D3(cholecalciferol).[3] Vitamin D3 is generally addressed as vitamin D, as it has higher contribution to increasing serum 25-hydroxy vitamin D levels.[3,4] It helps to maintain adequate calcium and phosphate levels, which are in turn required for muscle contraction, nerve conduction and mineralization of bone.[4] It serves a role in inflammation, cell multiplication and differentiation.[4] Vitamin D plays an anti-inflammatory role by inhibiting differentiation of Th17 cells.[5]

Various cut-offs for vitamin D have been used to define the risk of deficiency; however, most suggested cut-offs are usually 30 nmol/L, and stay in the 25–30 nmol/L range.[6] According to the cut-off suggested by WHO, an adult person is considered vitamin D deficient, if the blood levels of 25-hydroxyvitamin D are less than 27 nmol/L(10.817 ng/ml).[6] Many experts note that the thresholds might vary depending on a situation, and that other considerations, such as ethnicity, genetics, inflammation, age, calcium intake, and obesity should be taken into account while defining vitamin D status.[1]

Vitamin D levels are assessed by measuring the blood plasma levels of 25-hydroxyvitamin D.[7] The levels of 25-hydroxy vitamin D levels can be measured using high-performance liquid chromatography, chemiluminescence and radioimmunoassay.[7] Liquid Chromatography-tandem mass spectrometry is the most sensitive method used as the gold-standard :however, it is substantially more expensive than other methods.[7]

According to the Institute of Medicine (IOM), USA. it is recommended for children and adults in the US and Canada to intake about 600 IU of vitamin D per day.[8] The IOM has also suggested increasing the upper limit of intake from 2,000 to 4,000 IU per day.[8] According to IOM, the Estimated Average Requirement (EAR) of vitamin D is 400 IU per day for all age groups, while the Recommended Dietary Allowance for vitamin D is 1300 mg/day among 9-18 years old.[8]

The evidence suggests that the prevalence of vitamin D deficiency is the highest in Asia, the Middle East, and Africa, with young infants, pregnant and lactating women most affected.[6] The burden of vitamin D deficiency can also be measured by looking at the prevalence of rickets especially among children and early adolescents (<15 years).[6] Rickets prevalence is greatest in Asia, Middle East and Africa.[6,9]

Nutritional rickets in children is the main consequence of vitamin D deficiency in both high and low income countries.[6] The diseases associated with nutritional rickets include impaired linear growth, pneumonia because of deformed chest cavity, hypocalcaemia seizures, and cardiomyopathy. [6]Vitamin D deficiency in childhood and in-utero can cause retardation of growth and increase the risk of hip fracture and skeletal deformities.[6] In adults it leads to osteoporosis, muscle weakness and increases risk of fracture.[6] Age related osteomalacia, which is observed among older adults can be aggravated due to vitamin D deficiency.[6]

Vitamin D plays a role in reducing the chances of cancer autoimmune disease, infectious disease, and cardiovascular disease and some other chronic illnesses,.[9] Vitamin D deficiency has been shown in several studies to be associated with respiratory infections, asthma and tuberculosis.[6] Vitamin D deficiency can increase the risk of obesity, as well as diabetes mellitus, hypertension, and cancers of colon, breast, and prostate.[2] Also, literature reveals that higher vitamin D levels are associated with lower risk of Crohn's disease.[10]

Maternal vitamin D deficiency is also a prevalent problem.[11] Vitamin D deficiency seems to be associated with the risk of pre-eclampsia in pregnant women, which is defined by gestational hypertension and proteinuria that becomes normal after delivery. Women with vitamin D deficiency who are less than 22 weeks pregnant are at risk of developing pre-eclampsia; in addition, their children might develop low vitamin D levels.[11]

Risk factors for vitamin D deficiency

Exposure to sunlight

The main risk factor for vitamin D deficiency is inadequate exposure to sunlight.[12] Sunscreen usage (>SPF 30) can decrease vitamin D synthesis by 95% even under exposure to sunlight.[12] People with high melanin content in skin (dark skin) require high exposure for sufficient vitamin D synthesis.[12] Elderly people who tend to be indoors and are less exposed to sunlight are also among the risk group for vitamin D deficiency.[6] There are several factors that affect vitamin D synthesis in body through sun exposure which include season, latitude, time of day, air pollution level, skin tone, dressing, application of sunscreens and age.[13]

Obesity

According to the recent studies, obesity can decrease the bioavailability of vitamin D.[14] People with fat metabolism syndromes and bariatric patients are unable to properly absorb the fat soluble vitamin D.[12] Increasing Body Mass Index in females was associated with vitamin D deficiency in a study done among urban adults in Bulgaria.[15] It has been shown that obese adolescents tended to have lower levels of 25-hydroxy vitamin D compared to the children with normal weight.[16,17]

Genetic and Hereditary Factors

Evidence suggests that vitamin D deficiency can also be hereditary and associated with genetic factors.[18] The genetic mutations in gene GC, 25-hydroxylase gene (CYP_2R_1) and 7-dehydrocholesterol reductase gene ($DHCR_7$) were associated with plasma levels of 25-hydroxy vitamin D levels.[19] Polymorphisms in gene coding for vitamin D binding protein and vitamin D receptors were associated with the 25-hydroxy vitamin D levels in blood.[20]

Cigarette smoking

Cigarette smoking status has been found to be associated with vitamin D deficiency.[21,]It has been shown, that the exposure to cigarette smoke both in an active and passive manner seems to suppress the levels of cholecalciferol (vitamin D3) and calcitriol(Calcium).[22] Several pathways have been suggested, including decreasing the vitamin D absorption by cigarette smoke.[22] Calcium plays a role in vitamin D metabolism in the body and thus might affect the level of vitamin D.[23,24] It has also been noted that Vitamin D deficiency might aggravate the effects of smoking in lungs compared to the subjects who are not vitamin D deficient.[25]

Socio-economic status

Vitamin D deficiency is found to be more prevalent among people of lower socio-economic status. [26,27] The possible explanation is that people with lower socio economic status might consume the products containing vitamin D (i.e. dairy products) less often than those with higher socio economic status, which makes the lower socio economic group more prone to vitamin D deficiency.[28]

Diet

Dietary intake of vitamin D is essential in adequate amounts even in the presence of sufficient sun exposure and healthy weight.[29] Vitamin D rich sources include mostly dairy products and fatty fish.[2]

Studies have shown that inclusion of food items such as low fat gouda cheese in diet, has increased the amount of 25-hydroxy vitamin D levels among the post menopausal women.[30] The fast food consumption was observed to be negatively associated with the levels of 25-hydroxy vitamin D in plasma among adolescents.[31] Increased soft drinks consumption has also been reported to be associated with vitamin D deficiency among adolescents and premenopausal women.[32,33] The soft drinks contain phosphate that inhibits renal 1α hydroxylase that further reduces 1α,25-hydroxy vitamin D levels.[34] Also, the acid content reduces calcium levels in the body.[34]

Physical activity

Engaging in physical activity has been shown to increase the levels of 25-hydroxy vitamin D levels.[35] Among adolescents physical activity scores were directly positively associated with the levels of vitamin D deficiency.[31] Physical activity increases local bone mass, raises efficiency of absorption and reduces calcium excretion, by which it helps maintain the vitamin D levels in body.[36] At the same time, vitamin D deficiency among adolescents, especially girls seemed to produce muscle fatigue and decreased motivation to get involved in exercise programs.[37]

Gender

Some studies show that vitamin D deficiency is more likely to be found among females.[38] For example, in the study conducted in Kuwait in 2016 vitamin D deficiency was more prevalent among adolescent girls compared to adolescent boys.[39] Similarly, Jung So Lim et.al study reported higher prevalence of vitamin D deficiency among healthy women than in men.[40] A study done in a rural area of India, among adolescents aged 10 to 20 years revealed that the prevalence of vitamin D deficiency was higher among girls compared to boys, which was explained by presumably better diet provided to boys in the households and higher level of

sun exposure.[41] However, it has also been noted, that boys need higher amount of vitamin D for their skeletal modelling phase.[40] A study conducted in 2016 in India among adults aged above 25 years found higher prevalence of deficiency among males. Further studies are necessary to explore the relationship between vitamin D deficiency and gender.[42]

Medical conditions associated with vitamin D deficiency

People with nephritic syndrome are prone to lose 25-hydroxy vitamin D binding protein in urine.[12] People who have granuloma-forming disorders and lymphomas are also at risk of developing vitamin D deficiency. In patients with hyperparathyroidism the metabolism of 25-hydroxy vitamin D is increased and thus they are at risk of vitamin D deficiency.[12] People suffering from chronic autoimmune atrophic gastritis are more likely to be vitamin D deficient due to decreased absorption.[43]

Age

Older age was associated with lower levels of vitamin D in plasma as older people tend to spend more time indoors and thus lack the exposure to sunlight.[44] The other factors that might contribute to vitamin D deficiency prevalence in older age include the decline in 7-dehydrocholesterol in skin which is a precursor for vitamin D, and increased fat deposition that contributes to large distribution volume for storage of 25-dehydroxy vitamin D and the subsequent decrease in bio availability.[45]

Vitamin D supplementation

It has been reported that daily intake of vitamin D supplements along with calcium increased the levels of 25-hydroxy vitamin D levels in plasma compared to the individuals who do not use the supplements.[46] A dose of 100 IU of vitamin D daily increases the serum 25-hydroxy vitamin D level by 1 ng/mol (2.5 nmol/l).[47]

It has been found that the bioavailability of both calcium and vitamin D is enhanced when they are taken together rather than separately.[23]

Situation in India

Several studies have reported that the prevalence of vitamin D deficiency among the general population of India is ranging from 50% to 94%.[48] Protein binding radioligand assay was used in one of the studies done in southern India for assessing the plasma 25-hydroxy levels in blood.[49] Chemiluminescence assay was also used to assess the 25-hydroxy vitamin D levels in plasma.[50]

The increase in vitamin D deficiency in India in the recent years might be explained by lifestyle changes, such as spending more time indoors than outdoors and decrease in physical activity.[48] The problem is particularly prominent in urban areas where indoor lifestyle and less sunlight exposure is common.[48] Other factors include pollution that decreases the haze scores of UVB rays[51]; low vitamin D and calcium diet[48]; fibre rich diet that contain phylates and phosphates which can decrease vitamin D[48]; cultural practices such as wearing purdah, clothing that reveals less amount of skin[48], and pregnancies with short intervals that do not allow the replenishment of vitamin D levels.[48]

Obesity, which has been shown to be associated with vitamin D deficiency, is highly prevalent in India, especially in South India.[52,53]

A study done in Tirupathi, south India, revealed that the vitamin D synthesis was at maximum when an individual was exposed to sun from 11 a.m. to 2 p.m.[13] To be vitamin D sufficient, a minimum of 3 days a week of exposure of arms and legs with 0.5 minimal erythemal dose of sunlight is required.[13] However, it depends on the season, location(latitude), clothing, skin pigmentation e.t.c,[13] In New Delhi, it was observed that for

adequate vitamin D levels, 10 minutes to half an hour sun-exposure of arms and legs from 10 a.m to 2 p.m is necessary.[54]

Vitamin D deficiency among adolescents

A meta analysis showed that the prevalence of vitamin D deficiency among the adolescent girls in India was 25.7%.[38] There is an increased demand for nutrients in the adolescence, as it is a rapid growth phase, with the demand being more pronounced among females as compared to males.[38] The reproductive changes happen in this period thus vitamin D deficiency can lead to Poly Cystic Ovarian Disease, endometriosis and problems in fertility.[38] 25-hydroxy vitamin D levels are also found to be associated with calcium and vitamin D intake in diet.[55] Also, vitamin D deficiency is more likely to affect the adolescent girls rather than boys because of the social practices including better nutrition provided to male children in some areas, and the restrictions on dressing laid on the female adolescents.[56]

Interventions to combat vitamin D deficiency

Several strategies have been used around the world in order to solve the problem of vitamin D deficiency. The main approaches have included fortification of foods with vitamin D and supplementation.[4] Supplementation as an intervention is not very efficient due to dosage and cost concerns.[4] Also, many people are not aware that they are vitamin D insufficient.[4] Fortification is considered a better alternative as it does not require people to be educated in order for them to take the intervention, and there are no dosage and cost issues.[4] The foods for fortification should be chosen according to the food product that are widely consumed in the country.[57]

It is recommended to consider edible oils for fortification because of the increased levels of their consumption , cost-effectiveness, and higher feasibility.[58]The World Food Programme has recommended to fortify palm oil, sunflower oil, flaxseed oil, and soybean oil.[58]

Rationale for the study

Few studies have been conducted in India to explore the prevalence of vitamin D deficiency and the factors associated with the deficiency among adolescents. As adolescence is accompanied by the rapid growth of bones and sexual development, vitamin D is particularly important in this age group .[37] The proposed study will help to identify the important risk factors for vitamin D deficiency among Indian adolescents and inform intervention strategies to combat the problem. Since many parents and adolescents are unaware about their vitamin D status, this study will help the participants to identify their status and get the treatment if needed.

Research objectives

1. To assess the prevalence of vitamin D deficiency among school age adolescents (13-17 years old) in Coimbatore, India.

2. To explore the association between lifestyle factors, including sun exposure, obesity, soft drink consumption, amount of vitamin D in diet, physical exercise, and second hand smoking and 25-hydroxy vitamin D levels among school age adolescents (13-17 years) in Coimbatore, India.

3. To explore the association between socio demographic factors (age, socio-economic status and gender) and 25-hydroxy vitamin D levels among school age adolescents (13-17 years) in Coimbatore, India.

Methods

Study population

The study population will be adolescent population of the city Coimbatore, Tamil Nadu. Coimbatore is the second largest city in Tamil Nadu after Chennai (capital of Tamil Nadu). In 2017 the population of Coimbatore was 1.89 million.[59] Coimbatore is an industrialized city of Tamil Nadu.[60] Eighty-eight percent of the population is involved in income generation[60] About 28% of the population is below the poverty line.[60]

According to the WHO, the adolescent population includes people in the age range of 10-19 years.[61] However, it is proposed to include only those between 13 and 17 years of age as the target population to ensure the feasibility and the logistical simplicity of the study.

The inclusion criteria for the students are the following:

(i) Students within the age group 13-17 years who study in the grades VII, VIII, IX, X and XI.

(ii) Students who can read and write in English or Tamil and have parents who can read and write in English or Tamil.

Study design

A cross-sectional survey will include blood sample collection in order to assess the 25-hydroxy vitamin D levels in blood (outcome) and the administration of a self-administered questionnaire that will measure sun exposure, vitamin D in diet, age, exposure to second hand smoke and soft drink consumption. The height and weight of the participants will be also measured in the survey. This study design is chosen based on the cost and time considerations.

Variables and Measures:

Independent variables: BMI(BMI= Weight/ Height^2)[62](Continuous)[63], Amount of soft-drink consumption per week (in litres, continuous)[32], Physical Activity score(continuous)[64], Sun-Exposure (categorical)[65], Vitamin D in diet(continuous)[66]⁶⁶, Socio-economic status(categorical)[63] ,Gender (binary), Age (continuous), Calcium and vitamin D supplements intake (binary)[32], and Second hand smoking exposure (categorical).[67]

Dependent variable: Vitamin D status(Deficiency/ No deficiency)(binary).[68]

Study instruments

The questionnaire will be pretested among 10 students of the same age group from a school not selected for the participation in the study.

The information regarding obesity will be obtained by calculating the Body Mass Index using height and weight of the participant obtained during the data collection phase by trained nurse interviewers.[44] The blood samples will be taken from the participants for testing the levels of 25-hydroxyvitamin D.[68]

Students' questionnaire (Appendix 1)

Sun-exposure Measurement

The validated Sunlight Exposure Measurement Questionnaire will be used in order to measure the sunlight exposure among the students.[65] The questionnaire was validated based on the comparison with the polysulfone dosimeter .[69] The polysulfone dosimeters are used for personalized measurement of UVB exposure.[69] Polysulfone is a photosensitive polymer that is sensitive to the wavelengths of UVB rays that are absorbed by the human skin.[70] The questionnaire will measure the type of clothing, use of hat or helmet and sunscreen use.[69]

The duration of exposure per day will also be measured.[69] The radiation is highest during the time 11AM to 3 PM and thus in the other time periods (7AM to 11AM and 3PM to 7PM), the radiation is 40% of that from 11AM to 3PM.[69] The estimated exposure duration from 7 to 11AM and 3 to 7PM will be converted to 40% and added to the duration of exposure from 11AM to 3 PM.[69] The estimation of the percentage of exposed skin will be done using the adapted Lund and Browder chart for burns assessment (Appendix 2).[71] Based on the duration of exposure in a day the participants will then be categorized into sunlight exposure< 1 hr/day (low exposure), sunlight exposure 1-2 hr/day (moderate exposure) and sunlight exposure > 2 hr/day (high exposure).[69] The outcome is the duration of exposure per day and is a categorical variable. Example of the scoring method is provided in the Appendix 2.

Physical Activity Questionnaire

The physical activity questionnaire will measure the physical activity over the past 7 days among adolescents.[64] The questionnaire is an adapted version of The Physical Activity Questionnaire-Adolescents and The Physical Activity Questionnaire-Children. The scoring will be done based on the manual. The physical activity will be measured in scores. The variable will be continuous.[64]

Vitamin D questionnaire

The questions about vitamin D dietary intake used in previous studies will be included in the self-administered questionnaire.[66] The frequency of consumption of each of the food items will be measured.[72] The intake of supplements of calcium, vitamin D and multivitamins will be measured by adapting questions from the short vitamin D questionnaire.[73] The summative score will calculated based on all food items. The variable is continuous.

Soft drink consumption

Soft drinks consumption will be measured by multiplying the frequency and amount of soft drinks consumption in a week.[74]

Second hand smoking exposure

The exposure to second hand smoking in home will be assessed with one question, adopted from the Global adult tobacco survey.[67] The variable will be categorical.

Parents' questionnaire (Appendix 3)

Socio-economic status and parental education

The socio economic status and the educational level will be measured using questions adapted from previous studies conducted in India.[75]

Supplements intake

The questions are adapted from the short questionnaire for the assessment of dietary vitamin D intake.[73] Questions about vitamin D supplements, Calcium supplements and fish oil intake will be asked. The outcome variables are binary.

Sample size calculation

For the sample size calculation, comparing two sample proportions formula was used

$$n = \frac{z_{1-\alpha/2}\sqrt{2P'(1-P')} + z_{1-\beta}\sqrt{P_1(1-P_1) + P_2(1-P_2)}}{(P_1 - P_2)^2}$$

$P' = P_1 + P_2/2$

P_1 = estimated proportion (larger)

P_2 = estimated proportion (smaller)

n = sample size

α = level of significance

β = Type II error (Power)

z = Z-value

From a report of prevalence of vitamin D deficiency among adolescents, the prevalence among adolescent boys was 27% and that of adolescent girls was 42%.[76]

The z-value as 1.96. The level of significance will be 0.05 and power of 0.8.

Substituting the values in the sample size formula,[74]

$$n = \frac{(z_{1-\alpha/2}\sqrt{2P'(1-P')} + z_{1-\beta}\sqrt{P_1((1-P_1)+P_2(1-P_2))})^2}{(P_1 - P_2)^2}$$

$$P' = P_1 + P_2 / 2$$

$$P' = 0.42 + 0.27 / 2$$

$$P' = 0.345$$

$$n = \frac{\{(1.96)\sqrt{2(0.345)(0.655)} + (0.845)\sqrt{((0.42)(0.58)+ (0.27)(0.73)}\}^2}{(0.42-0.27)^2}$$

$$n = \frac{\{(1.96)(0.6723) + (0.845)(0.6638)\}^2}{0.0225}$$

$$n = (1.87861)^2 / 0.0225$$

n = 156.85

n = 157(approximate)

n is number of participants in one group. Thus the number of participants to be recruited for the study will be 314.

Adjusting for the design effect,

= 314 x 2

= 628.

Adjusting for refusal rate, 37.5%[31]

0.375 x 628 = 235.5

Adjusted sample-size = 628 + 235.5

= 863.5

= 864 (approximate)

The sample size for the study will be **864.**

Sampling strategy

The list of schools that contain both primary and higher secondary classes will be collected from the district Collectorate office. Multi-stage random sampling will be used to select the participants. Ten schools (clusters) will be selected randomly from the list using RANDBETWEEN command in Microsoft Excel. The list of students in each grade will be obtained from the principals. Eighty seven (864/10) students from each school will participate in the study. As the age group includes adolescents from 13 to 17 years old, the VIII, IX, X, XI and XII grades will be chosen. The number of students in each grade may

range from 30 to 70. The students will be chosen randomly from each grade. An equal number of 18 (87/5) students from each grade will be randomly selected. The random selection from each grade will be done using RANDBETWEEN command in Microsoft Excel.

The consent forms for parents will be provided to the selected students of standards or grades VIII, IX, X, XI and XII (age 13-17 years) and will be given a week time for their parent's acceptance or denial for their child to participate in the study. An oral assent before the participation will be obtained from the students.

Data collection

After the random selection of the schools, a week will be allotted to go to each of the selected schools and obtain permission for the study. The administrative heads of the school will be explained the importance and the rationale for the study and the methods of data collection. Qualified nurses will be hired for data collection to measure height, weight and collect blood samples. The data will be collected in the respective schools.

Steps of data collection:

1. Providing the consent forms in an envelope and the parent's questionnaire in another envelope to the parents of selected participants.

2. Obtaining two envelopes of the consent and the filled parent's questionnaire.

3. Administration of oral assent to the students.

4. Distribution of the self-administered questionnaires to participants and collection of filled in questionnaires

5. Measurement of height and weight by the nurses. Writing down the measurements in the questionnaire.

6. Collection of blood sample by nurses (see a detailed description on the steps in the Appendix 4).

Data entry and analysis

The participant ID will be of seven digits, with the first two digits signifying the number assigned to the school (out of 10). The next two digits will depict the grade of the student. The last three digits will show the participant number. The data entry and analysis will be done using the SPSS 21 software. Double data entry and data cleaning will be done. Simple and multiple binary logistic regression will be used to explore bivariate and independent associations of variables with the outcome. Multi co-linearity will be checked before doing the multiple regression.

Budget and timeline

The budget for the study includes the costs of human resources, data collection charges, transportation charges and incentive charges (Appendix 5). The budget sums up to be 17,760 USD. Blood sample collectors, statisticians, field monitors, and data entry technicians will be hired. The cost of equipment required for blood sample collection is included as well. The incentives include consultations with health care providers and provision of free supplements. Appendix 6 presents the proposed project timeline.

Strength and limitations of the study

Most of the risk factors analysed in the study are modifiable by the individual with proper education, thus the study can help to initiate working interventions among the adolescent population in Tamil Nadu.

As blood samples are to be obtained, assent and consent gaining can be challenging and can lead to high refusal rate. Also, there is a possibility for recall bias as the participants will be asked questions regarding their vitamin D intake in diet which requires recall over few months. Soft-drink consumption and sun-exposure requires recall over the past week. The generalisability of the study is limited to adolescents from an urban area of Coimbatore.

Ethical considerations

The preliminary approval for the study from the International Review Board of the AUA has been obtained. The students will be given the parental consent forms (Appendix 7), which they will take home and obtain signature from their parents; a week time will be given to the parents for the consent. An oral assent (Appendix 7) will be taken from the participants prior to taking blood samples and before administering the questionnaire. The purpose of obtaining blood samples will be clearly explained before collecting data from the students and their parents.

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Appendix 1: Questionnaire for students

Prevalence of vitamin D deficiency and the factors associated with vitamin D deficiency among adolescents (13 and 17 years) in Coimbatore

Self-administered Questionnaire for 13-17 years old students

Participant ID- -- -- ---

Date of interview ___/___/_____ (dd/mm/yy)

Height - _____ (in cms).

Weight - _____ (in kilograms).

The following questions intend to measure your vitamin D intake in diet, sun-exposure in the past week, soft-drink consumption in the previous week, physical activity in the past week and second hand smoking exposure. Please answer the following questionnaire by circling around the option you choose.

Example: (1)

Please read the questionnaire carefully and answer the questions. You are free to skip any question that you feel is inappropriate. Circle one option for each question unless specified.

1. Date of birth ___/___/_____(dd/mm/yy)

2. Gender – 1) Male 2) Female

I. Sun Exposure Measurement:

The following questions are to measure the amount of sun exposure that you have in a week. Please circle the options chosen by you.
(In case of day to day variation, please enter average for a week)

3. For how long in a day are you under direct sunlight? (Mark only one)

1) < 15minutes

2) 15-30 minutes
3) 30-60 minutes
4) > 60 minutes

4. Duration and time of sunlight exposure (Mark only one per row)

	< 30 minutes	**30-60 minutes**	**1-2 hours**	**2-3 hours**	**3-4 hours**
7am – 11am					
11am-3pm					
3pm – 7pm					

5. What usually is the sleeve length of your dress when you are exposed to sunlight? **(Mark only one)**

1) Half sleeves
2) Full sleeves

6. Do you use helmet**? (Mark only one)**

1) Yes

2) No **(Skip to question 8)**

7. If yes, how does the helmet cover you?

1) Head only

2) Head and face

8. Do you use cap when you are outdoors or exposed to sunlight? **(Mark only one)**

1) Never

2) Sometimes

3) Always

II. Physical Activity:

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). This includes sports or dance that make you sweat or make your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing, and others.

Remember:

- There are no right and wrong answers — this is not a test.
- Please answer all the questions as honestly and accurately as you can — this is very important.

9. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark a tick in only one box per row.)

	No	1-2	3-4	5-6	7 times or more
Skipping					
Rowing/canoeing					
In-line skating					
Walking for exercise					
Bicycling					
Jogging or running					
Aerobics					
Swimming					
Baseball					
Dance					
Football					
Badminton					
Skateboarding					
Soccer					
Street hockey					
Volley ball					
Floor hockey					
Basketball					
Ice skating					
Cross country skiing					
Ice hockey					
Others(specify) _____					

10. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)? (Check one only.)

1) I don't attend physical education.
2) Hardly ever
3) Sometimes
4) Quite often
5) Always

11. In the last 7 days, what did you normally do *at lunch* (besides eating lunch)? (Check one only.)

1) Sat down (talking, reading, doing schoolwork)
2) Stood around or walked around
3) Ran or played a bit
4) Ran around and played quite a bit
5) Ran and played hard most of the time

12.In the last 7 days, on how many days right after school, did you do sports, dance or play games in which you were very active? (Check one only).

1) None
2) 1-time last week
3) 2 or 3 times last week
4) 4 times last week
5) 5 times last week

13. In the last 7 days, on how many evenings did you do sports, dance, or play games in which you were very active?

1) None
2) 1-time last week
3) 2 or 3 times last week
4) 4 or 5 times last week
5) 6or 7 times last week

14. On the last weekend, how many times did you do sports, dance, or play games in which you were very active? (Check one only.)

1) None
2) One time
3) 2-3 times
4) 4-5 times
5)6 or more times

15. Which one of the following describes you best for the last 7 days? Read all five statements before deciding on the one answer that describes you.

1) All or most of my free time was spent doing things that involve physical effort.
2) I sometimes (1-2 times last week) did physical things in my free time (eg. Played sports, went running, swimming, bike riding, did aerobics)
3) I often (3-4 times last week) did physical things in my free time.
4) I quite often (5-6 times last week) did physical things in my free time.
5)I very often (7 or more times last week) did physical things in my free time

16. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

	None	Little bit	Medium	Often	Very often
Monday					
Tuesday					
Wednesday					
Thursday					
Friday					
Saturday					
Sunday					

17. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)
    1) Yes
    2) No

    If Yes, what prevented you? _____

III. Soft-Drink Consumption:(Example- Cola, Fanta, Sprite)

18. On how many days in the past week have you consumed soft drinks? (Circle one option), ("0" means no days, "1" means "once a week", "2" means "twice a week", "3"means "thrice a week", "4"means four times a week, "5"means five times a week, "6"means six times a week and "7"means seven times a week.)

0 1 2 3 4 5 6 7

19. What was your average consumption, on the day of consumption of soft drinks***? (Please look at the pictures of the cola bottles to answer).***

1) 0.25 L
2) 0.33 L
3) 0.5 L
4) 1 L
5) 1.5 L
6) 2 L

IV. Second hand smoking:

The following questions will measure your exposure to second hand smoking.

20. How often does anyone smoke inside your home?

1) Daily
2) Weekly
3) Monthly
4) Never

V. Vitamin D intake:

21. How frequently are the following food items consumed by you? (Please mark the corresponding box).

Food type	Daily	Weekly	Monthly	Never
Fish	☐	☐	☐	☐
Meat	☐	☐	☐	☐
Milk	☐	☐	☐	☐
Curd (Yogurt)	☐	☐	☐	☐

Eggs, including egg yolk	☐	☐	☐	☐
Butter	☐	☐	☐	☐
White bread	☐	☐	☐	☐

Appendix 2: Scoring protocol

Scoring protocol for the Sun-exposure measurement questionnaire:

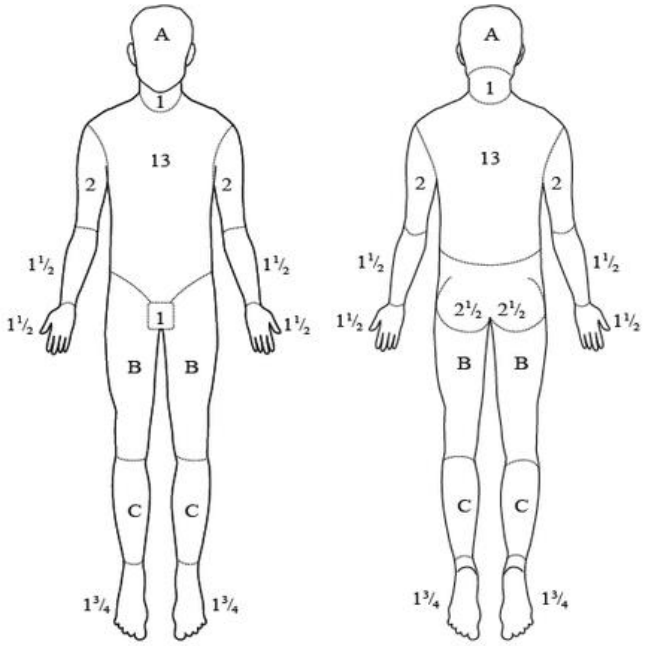
The duration of exposure is listed in various timing of the day,

a) The duration if from 11AM to 3PM is taken as such.
b) The duration from 7AM to 11AM and 3PM to 7PM is taken as 40%.

The skin exposure is measured using the Lund and Browder chart:

Therapeutic Guidelines

Lund and Browder chart for calculating the percentage of total body surface area burnt



Region	Partial thickness (%) [NB1]	Full thickness (%)
head		
neck		
anterior trunk		
posterior trunk		
right arm		
left arm		
buttocks		
genitalia		
right leg		
left leg		
Total burn		
NB1: Do not include erythema		

Area	Age 0	1	5	10	15	Adult
A = half of head	9½	8½	6½	5½	4½	3½
B = half of one thigh	2¾	3¼	4	4½	4½	4¾
C = half of one lower leg	2½	2½	2¾	3	3¼	3½

Example: A 12 year old participant was exposed to sunlight for 1 hour between 7AM to 11AM. The participant was wearing half sleeves, no helmet and a cap.

The duration per day would be,

1 hour x 0.4 = 24 minutes

Adjusting for the skin exposure from the chart,

6% (fore-arms) + 2% (neck) +5.5% (face) = 13.5%

The duration of the day would be,

0.135 x 24 minutes = 3.24 minutes.(< 1 hr/day)

Scoring protocol for the Physical Activity Questionnaire

Physical Activity Questionnaire-Adolescents

Scoring
Overall process - Find an activity score between 1 and 5 for each item (excluding item 9)
Five Easy Steps
1) *Item 1 (Spare time activity)*
- Take the mean of all activities ("no" activity being a 1, "7 times or more" being a 5) on the activity checklist to form a composite score for item 1.

2) *Item 2 to 7 (PE, lunch, right after school, evening, weekends, describes you best)*
- The answers for each item start from the lowest activity response and progress to the highest activity response
- Simply use the reported value that is checked off for each item (the lowest activity response being a 1 and the highest activity response being a 5).

3) *Item 8*
- Take the mean of all days of the week ("none" being a 1, "very often" being a 5) to form a composite score for item 8.

4) *Item 9*
- Can be used to identify students who had unusual activity during the previous week, but this question is **NOT** used as part of the summary activity score.

5) How to calculate the final PAQ-A activity summary score
- Once you have a value from 1 to 5 for each of the 8 items (items 1 to 8) used in the physical activity composite score, you simply take the mean of these 8 items, which results in the final PAQ-A activity summary score. 12

- A score of 1 indicates low physical activity, whereas a score of 5 indicates high physical activity.

Appendix 3: Questionnaire for parents

Gerald and Patricia School of Public Health

American University of Armenia

Prevalence of vitamin D deficiency and the factors associated with vitamin D deficiency among adolescents (13 and 17 years) in Coimbatore.

Parent's Questionnaire

Participant ID: -- -- ---

1. Does your child take multivitamin supplements?

   1) Yes 2) No

   a) If yes, how many multivitamin tablets in a day? _____

2. Does your child take calcium supplements?

   1) Yes 2) No

   a) If yes, how many IU per day? (As mentioned in the container)

_____

3. Does your child take vitamin D supplements?

   1) Yes 2) No

a) If yes, how many IU per day? (As mentioned in the container) _____

4. Does your child take cod liver oil or fish oil?

    1) Yes 2) No

5. Please indicate the child's mother's level of education

    1. Pre-primary

    2. Primary

    3. Secondary

    4. High school

    5. Bachelor's degree

    6. Master's degree

    7. Doctorate degree

6. Please indicate the child's father's level of education

    1. Pre-primary

    2. Primary

    3. Secondary

    4. High school

    5. Bachelor's degree

    6. Master's degree

    7. Doctorate degree

7. How would you rate your family's general standard of living?

    1) Substantially below average.

    2) Little below average

    3) Average

    4) Little above average

    5) Substantially above average

Appendix 4: Blood sample collection

The consent from the parents of each student will be obtained in a week prior to data collection. The selected students will be explained the reason for collecting the blood samples and blood samples will be taken only upon the student's permission. The blood sample to be collected requires a fasting blood sample. Thus the time of blood collection will be in the morning before their classes start. Trained nurses will do the blood sample collection, using the WHO guidelines for blood sample collection from vein.[77] The steps would include, assembling the equipment, preparing the person, selecting the site for blood collection, wearing gloves, disinfect the entry site, collect blood, filling blood in sample tubes, draw samples in correct order and labelling, cleaning contaminated areas, prepare samples for transportation and cleaning up of spills of blood or bodily fluids.[77]

1. The equipment required for the process are, sterile plastic tubes with rubber caps, non-sterile gloves, needles and syringes, a tourniquet, alcohol hand rub, disinfectant solution (70% alcohol rub), gauze or cotton balls, laboratory labels and transportation bags and containers.[77]
2. The participant will be first asked for their full name and will be asked again if they would want to give the blood sample.[77] If the participant agrees then he/she will be asked if they have allergy or phobias related to blood sample drawing and injections. The participant will be reassured and made comfortable by the nurse and will be asked to sit in a supine position. A towel or cloth will be placed below the arm of the participant. The nurse will make sure the participant has understood the procedure and verbal assent will be obtained.[77]
3. The ante-cubital region of the forearm is inspected for the vein.[77] A tourniquet is applied over 5 fingers gap above the identified vein and the vein is visualised again.[77]

4. The nurse will make sure their hands are washed well and they will wear gloves for the procedure.[77]
5. The site of blood collection will be disinfected using the 70% alcohol solution.[77] A swab of the solution will be used and done from centre to periphery.[77]
6. For the puncture, the thumb of the nurse in placed below the site of puncture of the vein, and the participant will be asked to clench his or her fist.[77] The needle will be entered at an angle of 30^0. After the blood is collected, the needle is removed and a cotton ball of gauze is placed over the punctured area.[77]
7. The laboratory glass tubes are filled with the collected blood and then sealed.[40] They are labelled with the participant ID. The tubes should be filled slowly to avoid haemolysis.[77]
8. The syringes, needles and cotton swabs used are disposed and the labels are rechecked before the participant leaves.[77]
9. The tubes are placed on a padded holder for safe transportation and to avoid breakage.[77]
10. The place is cleaned up if there are any blood or body fluids spill.[77]

    The collected blood samples will then be safely transported to the laboratories for checking the 25-hydroxy vitamin D levels.[77]

Appendix 5: Budget

Budget Item	**Type of Appointment**	**Number of Units**	**Amount per person /day (INR)**	**Number of days**	**Total INR**
Nurses	Appointed for the duration of process of data collection.	12	500	15	90000
Field monitor	Appointed for the duration of process of data collection.	2	300	15	9000
Data entry staff	Appointed at beginning of the data collection	2	500	20	20000
Statistician	Appointed after data entry	1	1000	15	15000
Total					134000

Materials for data collection:

Budget Item	**Number of Units**	**Amount per unit**	**Total INR**
Stadiometer	4	900	3600

Weighing scale	4	1000	4000
Syringes	870	2	1740
Needles with cap	870	3	2610
Cotton rolls(large)	50	100	5000
70% alcohol solution (470 ml)	40	2210	88400
Gloves	870	10	8700
Sterile plastic tubes with rubber caps (72 in a box)	10	9100	91000
Laboratory labels(sheets)	100	230	2300
Blood bank refrigerator	1	25000	25000
Laboratory testing charge	870	500	435000
Questionnaire printing	870	10	8700
Pen	50	10	500
Total			676550

Transportation charges:

Type of vehicle for rent	**Amount per day(INR)**	**Number of days**	**Total amount(INR)**
Van (25 seater)	2000	30 days(approximately)	60000

Incentive charges:

Type of service	**Amount per service**	**Number of participants**	**Total amount(INR)**
Consultation from health care provider	200	870	174000
Calcium and Vitamin D supplements	300	870	261000
			435000

Administrative charges:

Budget item	**Amount per month(INR)**	**Number of months**	**Total amount(INR)**
Office room	10000	5 months	50000
Office supplies	5000	5 months	25000
Total			60000

Total budget for the study: 1231550 INR. (17760 USD)

Appendix 6: Timeline for the study

Tasks	1 month	2 month	3 month	4 month	5 month
Obtaining list from collectorate office	**X**				
Hiring staff	**X**				
Pre testing of questionnaire	**X**				
Selection of schools	**X**				
Selection of participants	**X**				
Providing the consent forms		**X**			
Data collection		**X**			
Data entry and cleaning		**X**	**X**		
Data analysis			**X**	**X**	**X**
Preparation of final report				**X**	**X**

Appendix 7: Consent and Assent forms

Gerald and Turpanjian School of Public Health

Institutional Review Board#1

Written Consent Form for Parents (Adolescents aged 13-17 years)

Principal Investigator: Tsovinar Harutyunyan, MPH, PhD.

Co-investigators: Aida Giloyan, MPH.

Student investigator: Vanaja Dhanumoorthi, MPH Candidate.

Prevalence of vitamin D deficiency and the factors associated with vitamin D deficiency among adolescents in Coimbatore.

Hello I am Vanaja Dhanumoorthi. I am a graduate student from the Master of Public health course of the American University of Armenia. The School of Public Health of American University of Armenia is conducting a study to find the prevalence of vitamin D deficiency and risk factors associated with vitamin D deficiency among the adolescents in Coimbatore.

The study aims to find the prevalence of vitamin D deficiency and asses the associated risk factors for vitamin D deficiency among adolescents of age group (13-17 years in Coimbatore). Your child has been approached for participation in the study. Your child is the one out of 738 students, who was selected randomly

The study requires obtaining blood samples from the child to measure vitamin D level. The blood sample will be collected by trained nurses. This will help us to know whether your child is vitamin D deficient or not. Also, if your child is found to be vitamin D deficient then a free consultation to a health care provider and supplements will be provided to your child.

The results of the blood analysis will be sent to your child, in an envelope after a month of data collection.

Your child's height and weight will be measured by nurses. A questionnaire will have to be filled by your child in for assessing the lifestyle factors that are associated with the vitamin D levels in blood. The questionnaire will measure the vitamin D intake in your child's diet. It will also measure the soda drink consumption by your child over the week, physical activity of your child in a week and sun light exposure in a day. You as a parent are required to fill the questionnaire attached to this form if you agree for your child's participation. The time to administer the questionnaire would be 20 minutes, 5 minutes to measure height and weight; 10 minutes for blood sample collection. The duration of participation in the study would include 35 minutes. Your child will be contacted twice if he/she is participating, once for data collection and then for providing the blood test results.

Your child's participation is voluntary. Participating or not participating in this study will not affect your child's marks/grades in any manner. You and your child are free to withdraw from the study at any point and it will not have any negative consequences for your child.

The name of your child will be obtained in order to provide the blood test results after a month of blood sample collection. The information that we collect in the study will be used only for research purposes. The data collected will be accessed only by the research team and only the generalized findings will be included in reports and presentation. This research will help us in knowing whether the adolescents in Coimbatore are vitamin D deficient and if so, what are the lifestyle practices we can change for solving the problem and improving the situation.

If you have any doubts regarding the study you can contact our primary investigator of the study, Dr. Tsovinar Harutyunyan, MPH, PhD (tsovinar@aua.am). If you feel like your child has been mistreated or harmed while participating in the study, you can contact our Human Participant Protections Administrator, Varduhi Hayrumyan(auairb@aua.am).

If you agree with your child participating in this study, kindly sign below. Also please answer the questions below which will help us to understand the factors affecting the level of vitamin D in children. You are free to skip any question that you feel is inappropriate from the questionnaire. The filled questionnaire and this form are to be sealed in an envelope provided and sent along with your child to the school. Please send the consent form and the filled questionnaire in two separate envelopes.

Name of the Parent/Guardian:

Signature:

Date:

Oral assent for children:

Gerald and Turpanjian School of Public Health

Institutional Review Board#1

Oral Assent Form for Students (Adolescents aged 13-17 years)

Principal Investigator: Tsovinar Harutyunyan, MPH, PhD.

Co-investigators: Aida Giloyan, MPH.

Student investigator: Vanaja Dhanumoorthi, MPH Candidate.

Prevalence of vitamin D deficiency and the factors associated with vitamin D deficiency among adolescents in Coimbatore.

Hello, I am Vanaja Dhanumoorthi, a student from the School of Public health of the American University of Armenia. We are conducting a study to explore vitamin D deficiency among adolescents and the factors that affect it among adolescents of age 13 to 17 years in Coimbatore.

You are selected randomly from the list provided by the school. You are appreciated to either agree or disagree to participate in the study. Participating in the study will not affect your grades in any manner. Your participation is beneficial for the research which will in turn be useful for improving the health of the adolescents in Coimbatore.

We will need to obtain your blood sample as part of the study. The blood samples will be collected by the nurses here at school. This will help us to find the vitamin D levels in your body and decide whether you are vitamin D deficient or not.

You will also have to fill in a questionnaire in which you will have to give answers about your physical activity, diet and sun exposure. The nurses will measure your height and weight.

The information that we obtain from you will be used only for the purpose of the study. No identifiable information will be collected. The information obtained will be kept confidential.

Your participation is voluntary. You are free to skip any question in the study. You are free to stop your participation at any stage of the study. You are encouraged to ask any kind of questions that you have regarding the study.

Do you have any questions?

If you agree to participate, please stay in the room. Thank you for your attention.